INSTALLATION REQUIREMENTS FOR THE ALEXYS® NEUROTRANSMITTER ANALYZER WITH ACETYLCHOLINE SCC APPLICATION KIT

This document describes the details of the application specific chemicals, solutions and consumables required at the customer's site for a successful installation of the Acetylcholine application, based on the combination of the base system ALEXYS Neurotransmitters (p/n 180.0091U) and the Acetylcholine Application kit SCC (p/n 180.0506).

Other important documents that have to be taken into account before the installation are:

- Installation requirements ALEXYS FLEX/Neurotransmitter Analyzer (p/n 180.7070C)
- PC requirements (p/n 195.7000)

The customer must arrange the listed necessities in all these documents before the start of the installation. Therefore, these documents should be available to the customer well in advance of the installation date to be able to take the necessary actions.

Acetylcholine application kit

The application kit contains the column, cell, connectors and any other part that is necessary to run the application on the ALEXYS Neurotransmitter Analyzer (p/n 180.0091U).

The Acetylcholine application kit contains an enzyme based peroxidase kit and IMERs. These items are transported in a cooled box and need to be stored at 4°C immediately after receipt.

Laboratory facilities

- For the preparation of the LC solutions and standards, access to the proper facilities is a prerequisite (microbalance, pH meter and relevant pH standards, analytical pipettes, pipette tips, tubes, glassware such as measuring cylinders, etc.).
- An ultra sonic bath is required for degassing the mobile phase, the piston backwash solution and the auto sampler wash liquid before installation in the ALEXYS® system. **Do not use vacuum filtering units for degassing** or filtering because it can introduce electrochemically active contaminations. The ALEXYS® system is equipped with in-line filters and degassers.
- Wide mouthed bottle with desiccant to store opened bottles of ACh and Ch in the freezer (these solids are very hygroscopic).
- The following items should be available for flow cell cleaning: Soft & dry paper tissue (for instance Kleenex facial tissues), a squeeze bottle with acetone, a squeeze bottle with deionized water.

Application specific chemicals

Necessary chemicals for column/IMER care and to run the application

- Phosphoric acid (85% w/v in water)
- Acetonitrile
- Octane sulphonic acid sodium salt (OSA)
- Tetramethylammonium chloride
- Di-sodium ethylenediaminetetraacetic acid Na_2EDTA
- 50% w/w NaOH in water
- Acetic Acid
- Standards of Acetylcholine and Choline

Note: 'HPLC grade' water is not recommended for use with EC detection. Instead, use deionized water with a resistivity of at least 18 MOhm- cm and TOC free (<10 ppb).

Analysis of Acetylcholine and Choline

Table 1. Composition of the different solutions necessary for the analysis of Acetylcholine.

Solution	Composition	Remarks
Passivation and sterilization solution	1:3 diluted solution of HNO_3	Do NOT run this solution over the column or IMER
System flush solution	10% acetonitril (v/v) in water	Do NOT run this solution over the column or IMER
Mobile phase	50 mM phosphoric acid, 0.5 mM $EDTA.Na_2$, set to pH 7.5 with 50% NaOH solution, 1.6 g/L octanesulfonic acid, 0.5 mM tetramethylammonium chloride	For separation of ACh and Ch
IMER storage solution	0.1M Phosphate buffer, pH 8.2	Do NOT run this solution over the flow cell

Preparation of mobile phase

Preparation of 1L mobile phase with a composition as given in Table 1:

- Dissolve 0.186 g Na_2EDTA in about 900 mL demineralised water.
- Add 3.45 mL of 85% (w/v) solution H_3PO_4 to about 900 mL demineralised water.
- Calibrate a pH meter.
- Set the pH of the mobile phase to 7.50 ± 0.05 with 50% w/v NaOH in water
- Dissolve 1.6 g octane sulphonic acid
- Dissolve 0.0548 g tetramethylammonium chloride
- Fill up to 1 L with demineralised water
- Degas the mobile phases for 15 minutes in a sonic bath (Do not filter!).
- The mobile phase should be prepared fresh at least once a week.

Preparation of IMER storage solution

Preparation of 250 mL IMER storage solution:

- Add 1.71 mL of 85% (w/v) solution H_3PO_4 to about 230 mL demineralized water.
- Calibrate a pH meter.
- Set the pH of the mobile phase to 8.20 ± 0.05 with 50% w/v NaOH in water
- Fill up to 250 mL with demineralized water.
- Degas the solution for 15 minutes in a sonic bath (Do not filter!).
- The storage solution should be prepared fresh every time the IMER is stored.

Glassy carbon working electrode coating procedure

The analysis of ACh requires a peroxidase-coated glassy carbon electrode. An alternative is the platinum electrode, but this gives less sensitive results, and shorter stable working time.

The guideline below is adapted from the coating procedure described in the BASi peroxidase kit manual:

- Polish the glassy carbon disk for about 1 min on a polishing disk with a drop of 1 µm diamond slurry.
- Rinse the surface with distilled water and wipe off with acetone.
- Apply 3.5 µL surfactant on the GC WE and roll the solution evenly over de surface with the side of the syringe until dry.

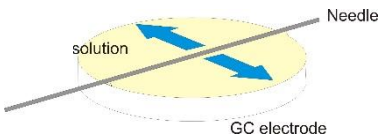

Fig. 1. How to spread a solution evenly over the electrode.

- Let the surface dry completely, which will take about 10 min
- Clean the syringe with distilled water (interior: by filling and emptying at least 10 times)
- Apply 7.5 µL peroxidase/polymer solution on the GC WE and use the side of syringe to make a uniform layer before it is completely dry. This can take about 10 minutes.
- Let the electrode dry further for 5-16 hours under an inverted beaker to shield it from dust.
- Clean the syringe with distilled water (interior: by filling and emptying at least 10 times)
- Install the electrode in the system or store it in a fridge at 4 °C

- **Do not disturb the surface after coating by touching or wiping.**
- **Coated electrode can be stored one week in the refrigerator at 4 °C**
- **A coated electrode can be used a couple of days in a flowing system**

When starting up the application, it is recommended to coat two electrodes at the same time, the second electrode being a backup that can be dry-stored in the fridge.

Glassy carbon working electrode regeneration procedure

- Polish the glassy carbon working electrode surface with 1 µm diamond slurry
- Rinse the electrode with water and wipe it dry
- Wipe the electrode with a tissue with some acetone
- The electrode is ready to have a new coating applied

System start-up

Steps	Remark
1. 50 mL H_2O 2. 50 mL 15% HNO_3 in water 3. 50-100 mL H_2O, until pH>3 4. 50 mL mobile phase 5. Connect the degasser, in-line filter, column and IMER. 6. Run mobile phase for about 10 minutes 7. Attach the flow cell 8. Run mobile phase for 2-5 minutes 9. Turn on the flow cell, and let the baseline stabilise for 1-2 hours	Flush out the system from the pump up to the injector valve. (both in INJ and LOAD pos.) Do not run the acid through the in-line filter nor degasser! Stabilise column and IMER in mobile phase Wet the polymer film Stabilise system

System stand-by

If the system will be used every day or every few days, leave the flow cell on, and recycle the mobile phase. However, fresh mobile phase should be prepared every week.

System shut down

- Coated electrodes should be stored dry in the refrigerator
- Flush IMER with 0.1M Phosphate buffer (pH 8.2) for 20 min at 130 µL/min
- Remove the IMER and put it in the shipping tube, containing fresh storage solution (0.1M Phosphate buffer, pH 8.2). Store in refrigerator at 4°C. DO NOT store it in the freezer. For detailed storage information see document 250.7014 shipped with the IMER.
- Pre-flush the analytical column with 10% acetonitril and subsequently with 100% acetonitrile. Read the column care and instruction manual of Waters for more details. This document can be loaded from the Waters website (try link below or go to www.waters.com).
  http://www.waters.com/waters/support.htm?lid=10008566&type=USRM

APPENDIX

A list of chemicals is shown below as a guideline for the purchase of chemicals by the end-user. The listed brands/purities are not necessarily the best chemicals, but the application was evaluated/developed at the Antec R&D laboratory using these specific brands/purities. If for any reason alternative chemicals need to be purchased use the following guidelines:

- The chemicals should have at least the same purity or better then the chemicals listed in the table below
- Do not purchase ultra dry grade or anhydrous chemicals

Table 2. Brands and purities of chemicals used for application development at Antec.

Component	Purity	Brand	Order no:	Mw	kg/L
Phosphoric acid, 85% w/v in water	puriss. p.a., ACS reagent	Fluka	79620	98	D:1.68
1-Octane sulphonic acid, sodium salt (OSA)	HPLC grade	Acros	384771000	216.28	
Tetramethylammonium chloride	puriss., p.a., for ion pairing chromatography	Fluka	74202	109.6	
Acetic Acid	For analysis, 99.8%	Acros	222140010	60.05	D:1.048
NaOH, 50% w/v in water	puriss., p.a., for HPLC	Fluka	71686	40.00	D:1.54
$Na_2EDTA.2H_2O$	SigmaUltra, 99%	Acros	147855000	372.23	
HNO_3	65% solution	Fluka	84380	63.01	D:1.40
Acetonitrile	HPLC grade, 99.9%	Acros	268260025	41.05	D:0.781
Acetone	General purpose grade	Fisher	A/0520/17	58.08	D:0.79
Methanol	HPLC gradient grade	Baker	8402	32.04	D:0.79
Sodium Acetate Trihydrate	Baker HPLC analyzed	Baker	0393	136.08	
Water	TOC <10ppb and deionised, resistivity >18 MOhm-cm (Barnstead Easypure II)				

Manufacturers

JT-Baker	http://www.avantormaterials.com
Sigma-Aldrich	http://www.sigmaaldrich.com
Fluka	http://www.sigmaaldrich.com
Fisher Scientific	http://www.fishersci.com
BASi	http://www.basinc.com
Barnstead	http://www.thermoscientific.com